Peptide stabilization by side-chain to side-chain cyclization

Maud Larregola and Anna-Maria Papini

PeptLab@UCP Platform
Laboratory of Chemical Biology
University of Cergy-Pontoise

Colloque Adebiotech – 24 septembre 2015

UNIVERSITÉ de Cergy-Pontoise

PeptLab UCP

Opportunities and Weaknesses in Developing Peptide Drugs

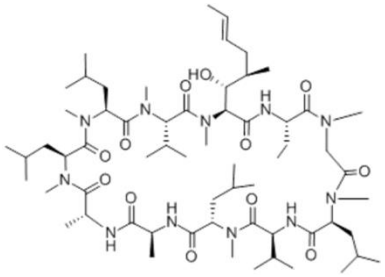
- Opportunities
  - Readily available leads
    - Synthesis and SAR are straightforward and rapid
  - A large number of diverse unnatural amino acids available to increase stability
  - Can access larger binding surface area than small molecules
    - Well suited for extracellular protein-protein interactions as GPCR agonist and antagonists
  - Possible to achieve high potency (sub-nanomolar) and efficacy
  - High selectivity and low toxicity

- Weaknesses
  - Peptide Therapeutics: It is all in the Delivery
  - High clearance: requires extensive optimization, fusion/conjugation and/or formulation

Strategies to Increase Peptide Half-Life

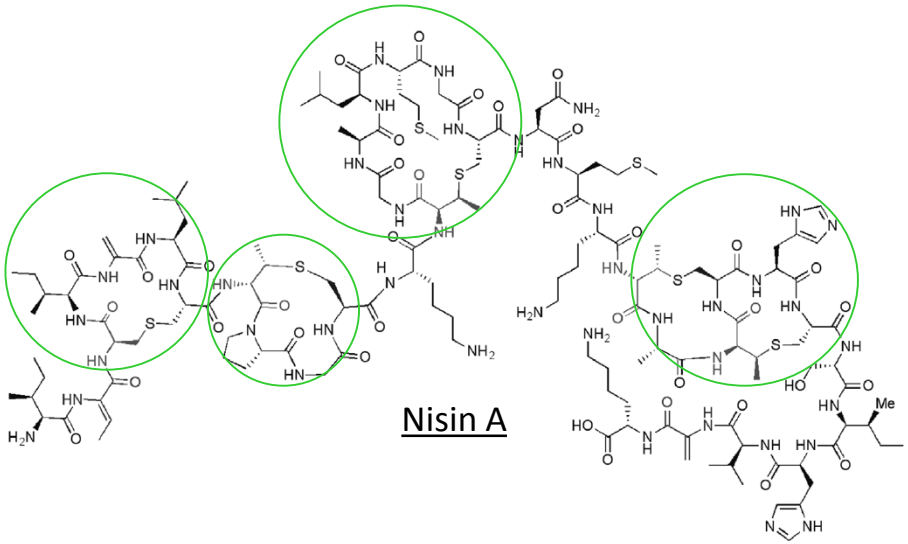
- Lipidation
  - Liraglutide (phase 3/registration) (Novo Nordisk)
- Pegylation
  - Hematide (phase 3, renal failure) (Affymax)
- Albumin Conjugation/Complexation
  - Albumin binding peptides (Genentech)
  - Domain Anti-albumin fusions (Domantis, GSK)
  - Albumin fusion proteins (Human Genome Sciences, GSK)
  - Covalent attachment (CJC-1411, Conjuchem)
- Antibody Conjugation/Complexation
  - Fc-fusions (Mimetibody, Centocor)
  - Ab-covalent attachment
  - Anti-digoxigen antibodies (Roche)

Cyclization: Learnings from Nature



Cyclosporin A: %F = 29

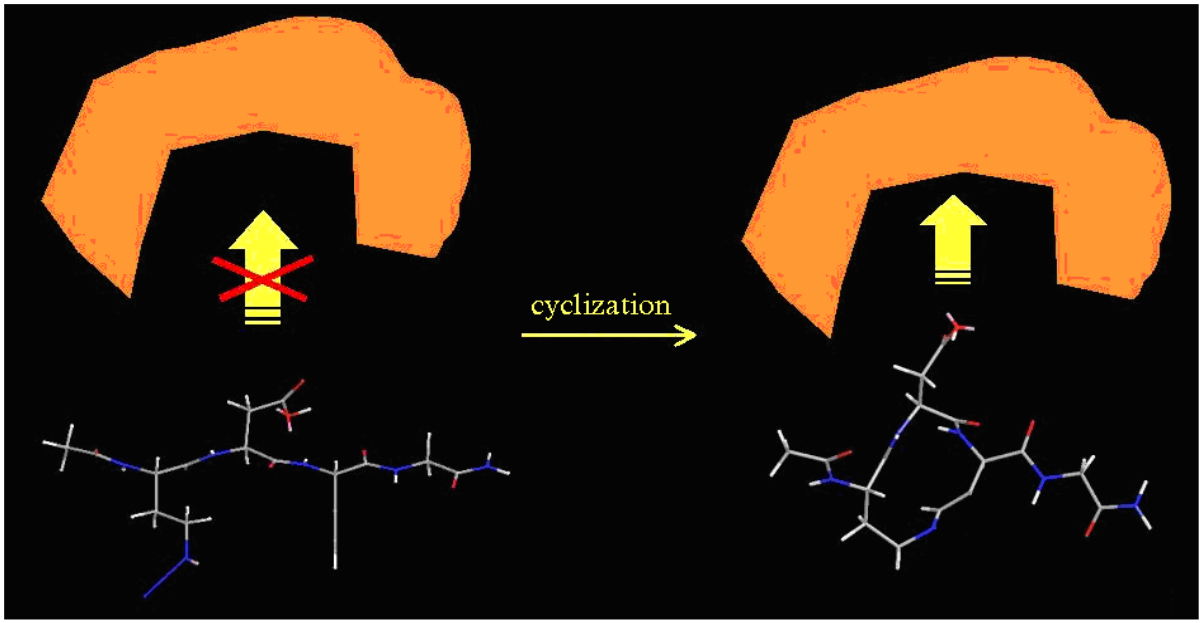
- orally bioavailable marketed cyclic peptide (11 aa)
- intramolecular H-bonds reduce desolvation penalty when leaving water



Nisin A

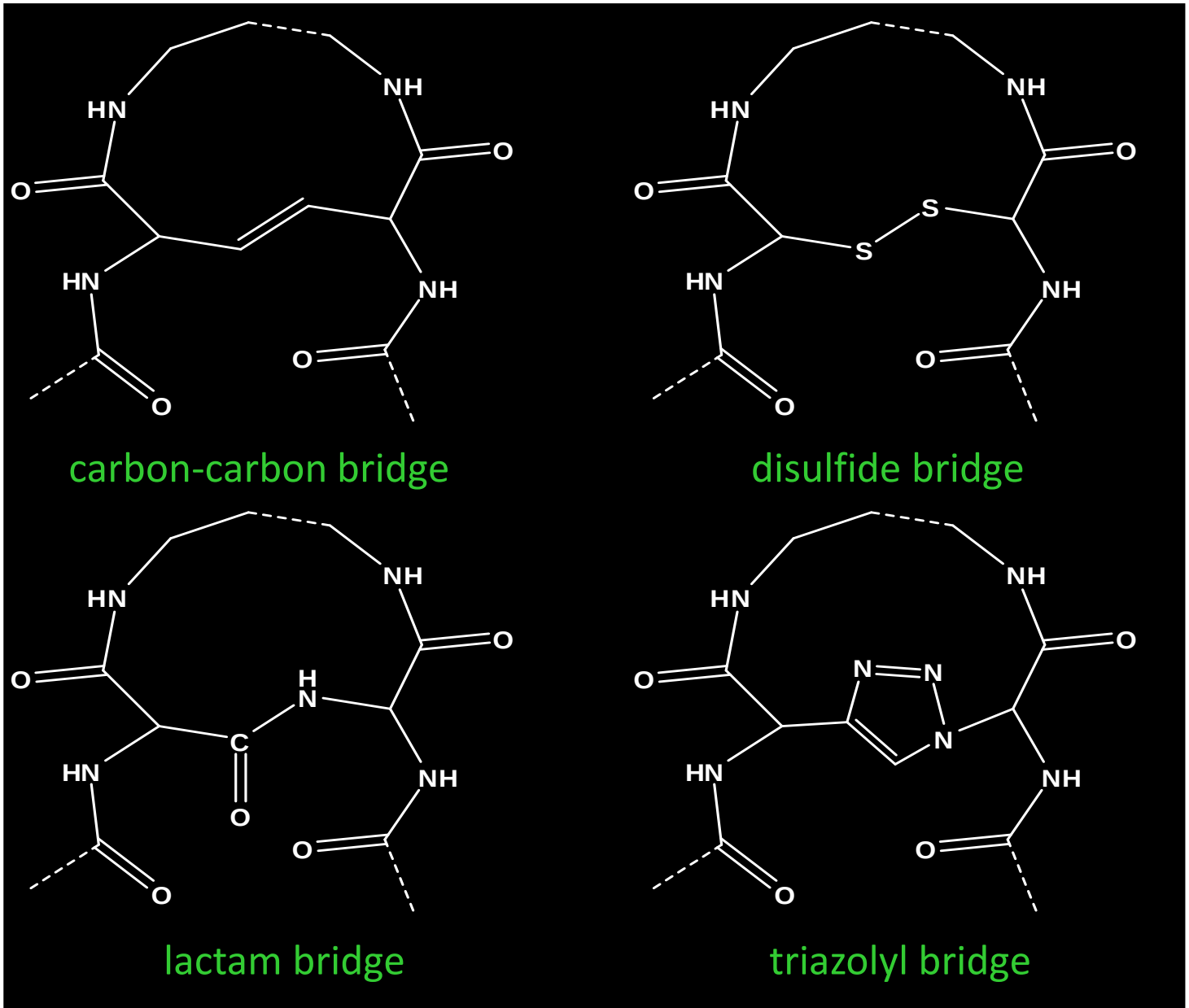
- Many intramolecular side-chain to side-chain cycles

Side-chain to side-chain cyclization

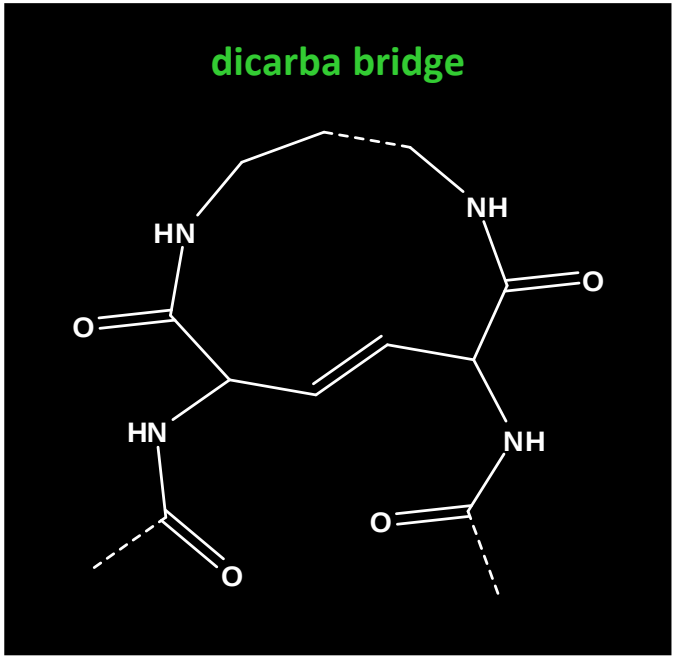


- Rigidification reduces susceptibility to proteolytic enzymes thus increasing the metabolic stability in vitro and more significantly in vivo
- Restriction to active conformation in cyclic peptides can give superpotent analogues in matched cases
- Basis for receptor selectivity: often different receptors bind the same flexible substrate in different conformations

Cyclization types

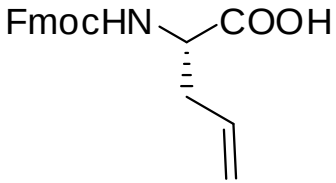


carbon-carbon bridge by Ring Closing Metathesis

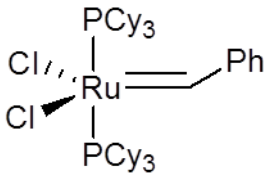


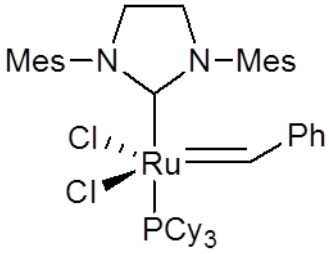
Starting material:

unnatural amino acids:
allyglycine



Cyclization:

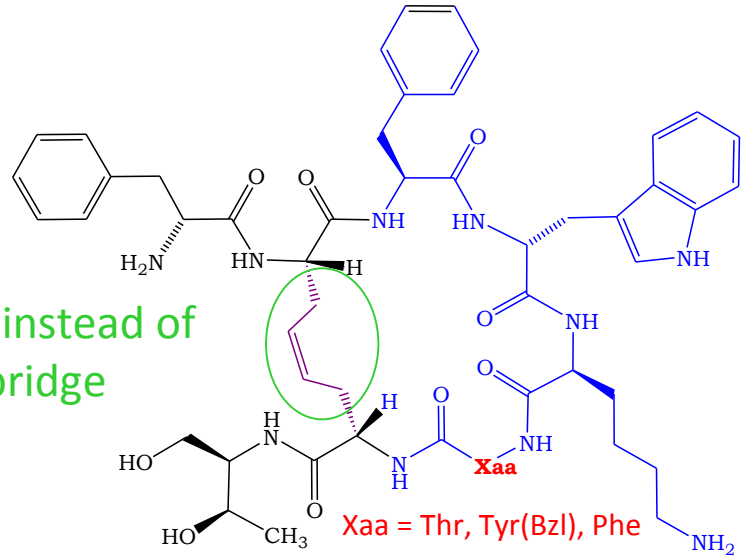




Presence of metal (Grubbs catalysts)

Dicarba-analogs of octreotide

- octreotide: octapeptide analog of the disulfide-bridged somatostatine hormone
- cell growth inhibitor in a few cancer types and carrier of radionuclides



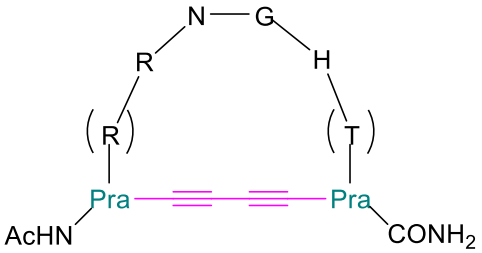
Dicarba group instead of disulfide bridge

Keeping pharmacophore region and type II' β-turn conformation

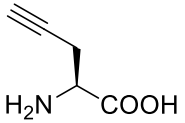
Increased stability (more than 30h in human serum)
Possible labelling with ^{99m}Tc and ^{188}Re (no disulfide cleavage in reducing medium)

Papini *et al.*, Letters in Organic Chemistry, **2005,** 2 No.3, 274-279.
Papini *et al.*, J. Med. Chem., **2008**, 51, 512-520.
Papini *et al.*, J. Med. Chem., **2010**, 53, 6188-6197.

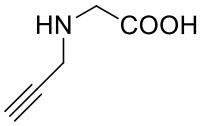
Another type of carbon-carbon bridge: 1,3-butadiyne



L-Pra: L-Propargylglycine



*N*Pra: *N*-Propargylglycine



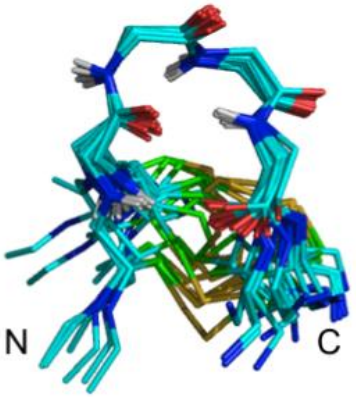
- Glaser oxidative coupling = a click reaction never explored to constrain peptide backbone
- catalyzed by copper(I) salt in the presence of oxygen (micro-wave assisted reaction)
- the diyne tether combines high rigidity and limited occupied space

Auberger N., Di Pisa M., Larregola M. *et al.*, Bioorg.Med.Chem. (2014) 22(24)

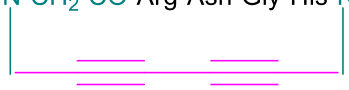
β-turn stabilization

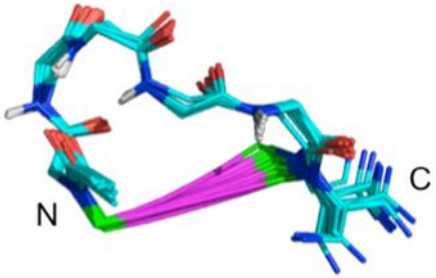
- including the minimal epitope RNGH for antibody detection in Multiple Sclerosis
- disulfide bridged hexapeptide / diyne bridged hexa or octapeptides
- NMR conformational analysis in water:

Ac-Cys-Arg-Asn-Gly-His-Cys-NH_2: I' β-turn centered on Asn-Gly

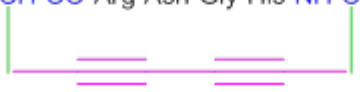


Ac-N-CH_2-CO-Arg-Asn-Gly-His-N-CH_2-$CONH_2$: I β-turn centered on Arg-Asn



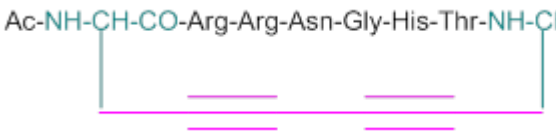


Ac-NH-CH-CO-Arg-Asn-Gly-His-NH-CH-$CONH_2$: no turn stabilization for the hexapeptide but…

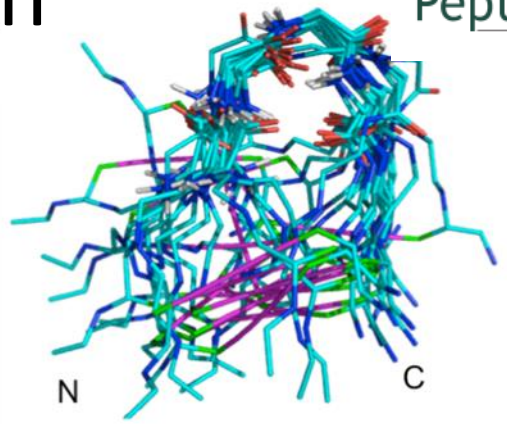


Auberger N., Di Pisa M., Larregola M. *et al.*, Bioorg.Med.Chem. (2014) 22(24)

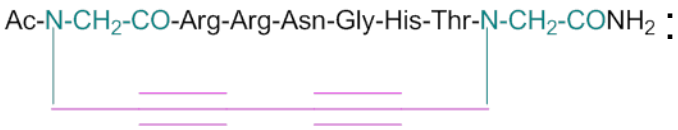
β-turn stabilization

…octapeptide 

:

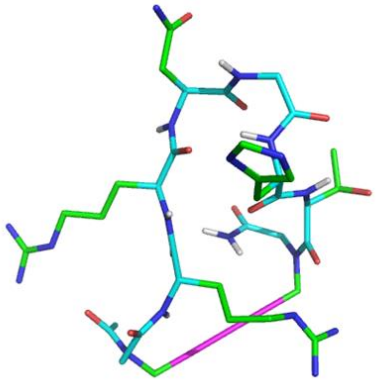


I' β-turn stabilization centered on Asn-Gly



:

β-turn structure around Asn-Gly



calculation without NMR restraints

- Diyne bridged cyclic peptides allow stabilization of various β-turn structures in water
- Optimization of on-resin Glaser oxidative coupling: libraries of stable constrained butadiyne peptides can be generated

Auberger N., Di Pisa M., Larregola M. *et al.*, Bioorg.Med.Chem. (2014) 22(24)

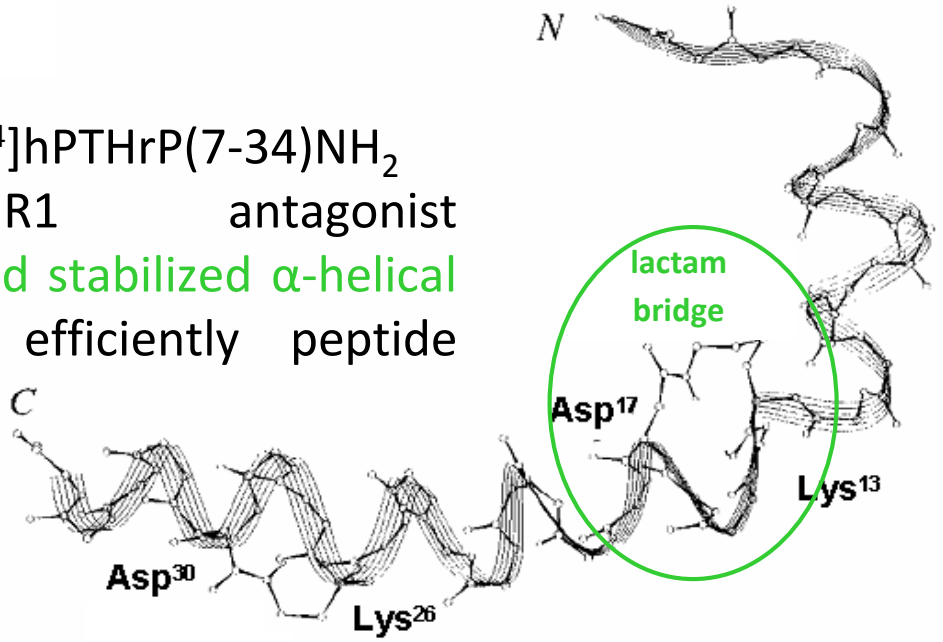
α-helix stabilization

- PTH = 84-aa hormone increasing Ca^{2+} concentration in blood
- PTHrP = 139-173 aa hormone causing humoral hypercalcemia of malignancy

- N-terminal portion essential for interaction with PTHR1 receptor

- Chorev *et al.* demonstrated that an α-helical motif is essential for the bioactive conformation:

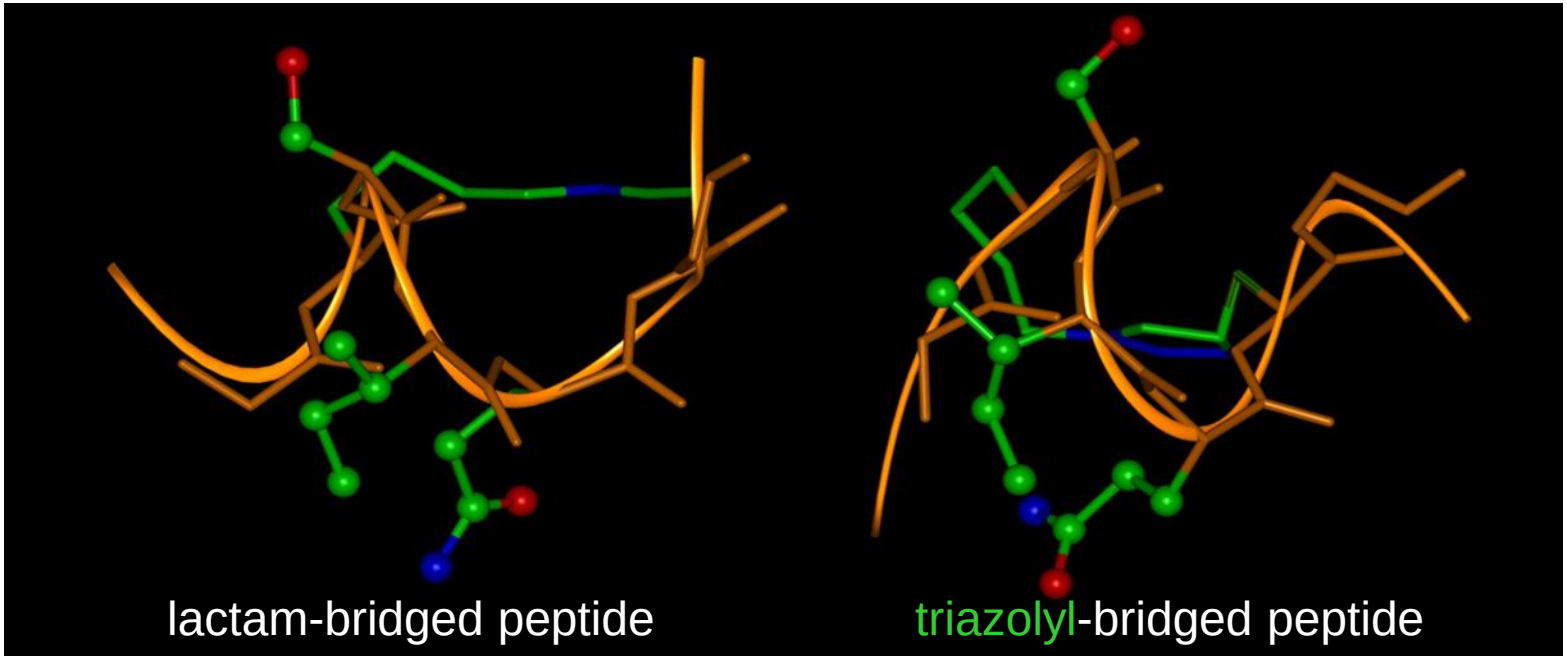
$[Lys^{13}(\&^1),Asp^{17}(\&^2),Tyr^{34}]hPTHrP(7\text{-}34)NH_2$
a potent PTHR1 antagonist containing an extended and stabilized α-helical conformation increasing efficiently peptide interactions



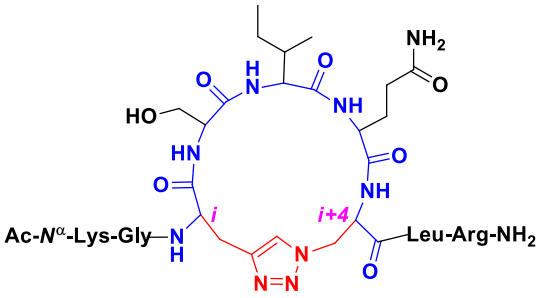
Schievano E.; Rosenblatt M.; Chorev M.; Peggion E. *J. Peptide Sci.* **1999**, *5*, 330-337
Bisello A.; Nakamoto C.; Roseblatt M.; Chorev M.; *Am. Chem. Soc.* **1997**, *36*, 3293-3299
Mierke D.F.; Bisello A.; Mammi S.; Peggion E.; Chorev M.; *Am. Chem. Soc.* **1997**, *36*, 10372-10383
Maretto S.; Rosenblatt M.; Chorev M.; Mierke D.F.; *Am. Chem. Soc.* **1997**, *36*, 3300-3307

α-helix stabilization

- NMR studies of Ac-hPTHrP(11-19)NH_2 derived cyclopeptides in water:HFA

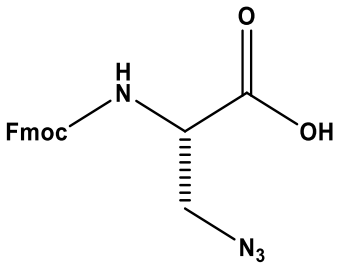


- α-helical structures in the cyclic part of the molecules
- slight difference of the backbone arrangement but common spatial orientation of side-chains

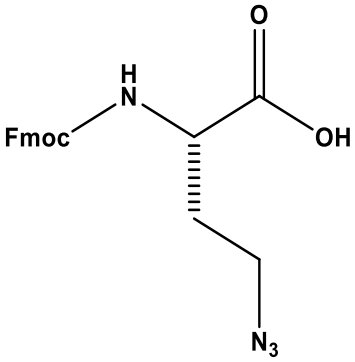


Papini *et al.*, J. Org. Chem. (2008) 73, 5663

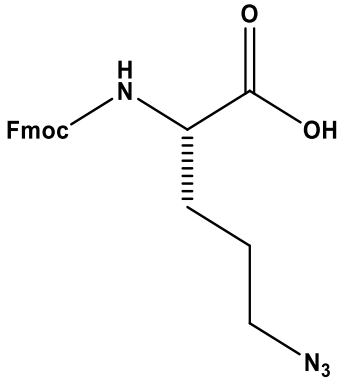
Unnatural amino acids for various azido-alkynyl intramolecular peptide cyclization



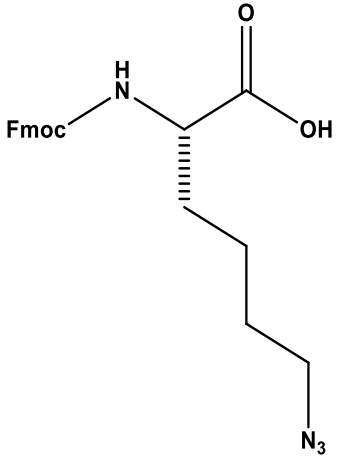
***N*$^{\alpha}$-Fmoc-Apr(β-N_3)-OH**



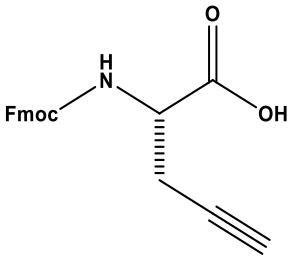
N$^{\alpha}$-Fmoc-Abu(γ-N_3)-OH



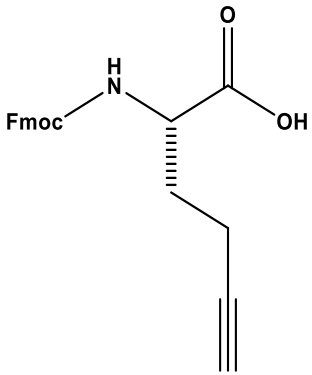
***N*$^{\alpha}$-Fmoc-Ava(δ-N_3)-OH**



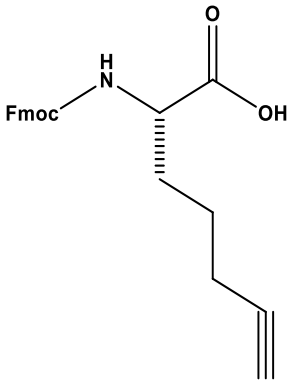
***N*$^{\alpha}$-Fmoc-Nle(ε-N3)-OH**



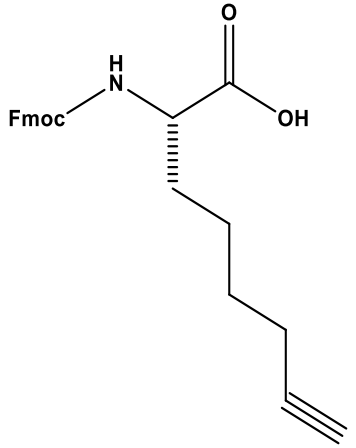
***N*$^{\alpha}$-Fmoc-Pra-OH**



***N*$^{\alpha}$-Fmoc-Abu(γ-yl)-OH**



***N*$^{\alpha}$-Fmoc-Ava(δ-yl)-OH**

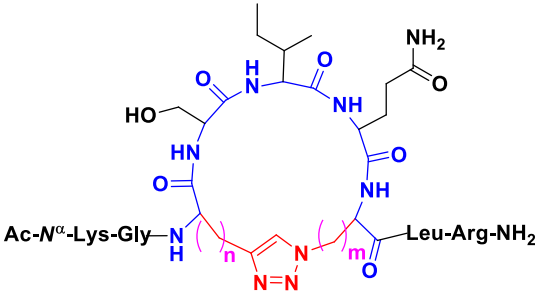


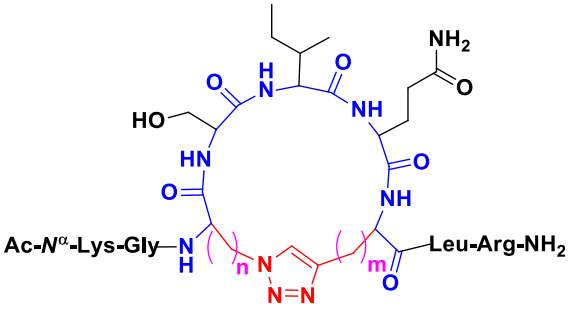
***N*$^{\alpha}$-Fmoc-Nle(ε-yl)-OH**

Papini *et al.*, *Eur. J. Org. Chem.* **2008**, 5308

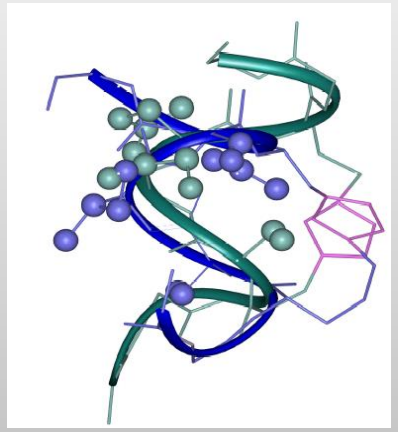
α-helix stabilization

- Variation in the size of the triazol-containing bridge, the location and orientation of the triazol in the bridge :

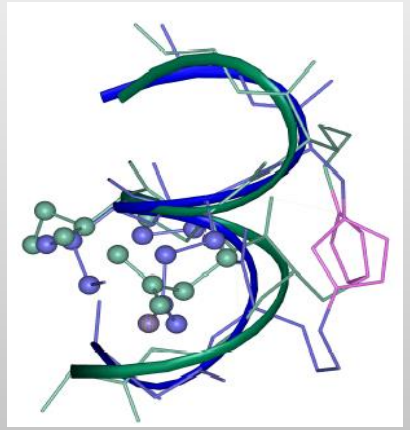




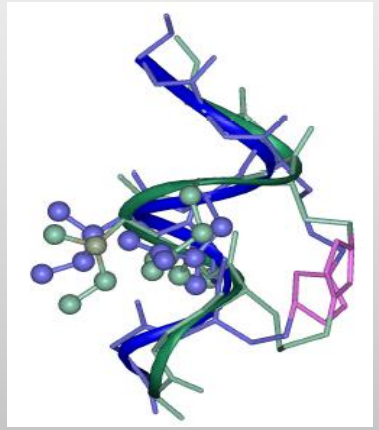
- NMR studies of Ac-hPTHrP(11-19)NH_2 derived cyclopeptides in water:HFA



4 methylene groups
Too short !

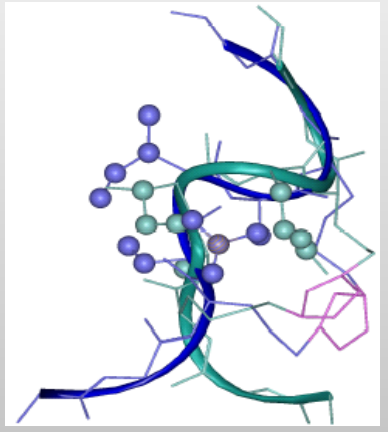


5 methylene groups



6 methylene groups

Good overlapping with original helical structure



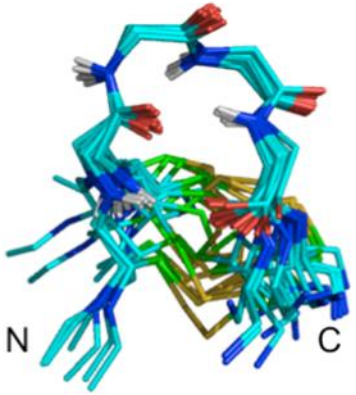
7 methylene groups
Too long !

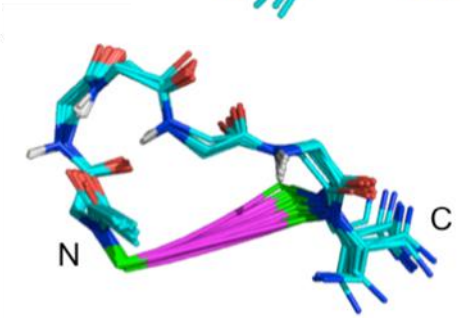
Papini *et al.*, *Eur. J. Org. Chem.* **2010**, 446-457

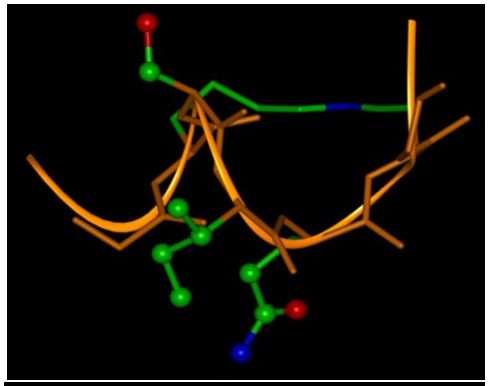
Peptide stabilization by side-chain to side-chain cyclization

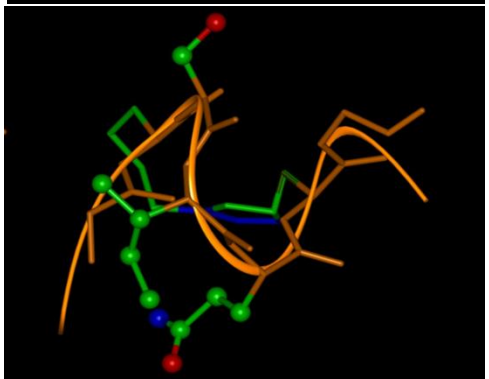
β-turn stabilization

- disulfide bridge
- dicarba-analog
- diyne tether









α-helix stabilization

- lactam bridge
- triazolyl bridge

PeptLab@UCP platform

- Created thanks to ANR chaire d'excellence Pepkit 2009-2014
- in Neuville-Université (RER A), Cergy-Pontoise
- Missions:
  - ✓ research, development or expertise services for industries or academics
  - ✓ Scientific equipment provision
  - ✓ Training courses in peptide synthesis

Equipment

Peptide synthesis

Biotage SyroWave™

Biotage Syro II

Liberty Blue **CEM**

Purification/ Characterisation

Autopurifier HPLC **Waters *2767***

UPLC-MS **Waters Acquity**

Equipment

Peptide-protein interaction analysis

TECAN (ELISA)

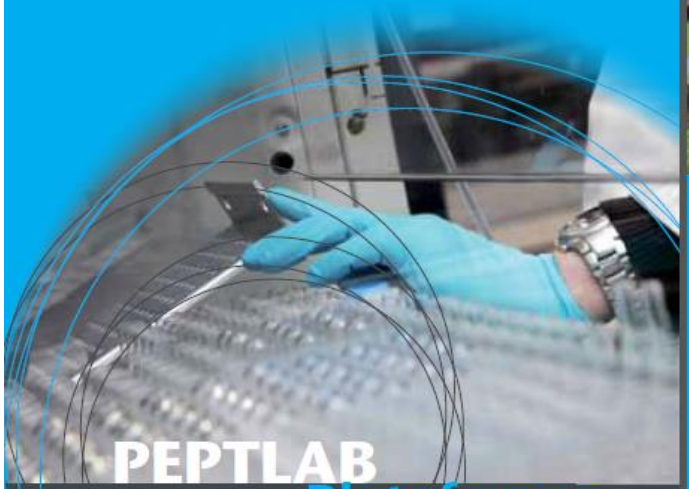
Surface acoustic wave
SAW intruments SamX

Microcalorimetry
GE Healthcare ITC200

UNIVERSITÉ
de Cergy-Pontoise

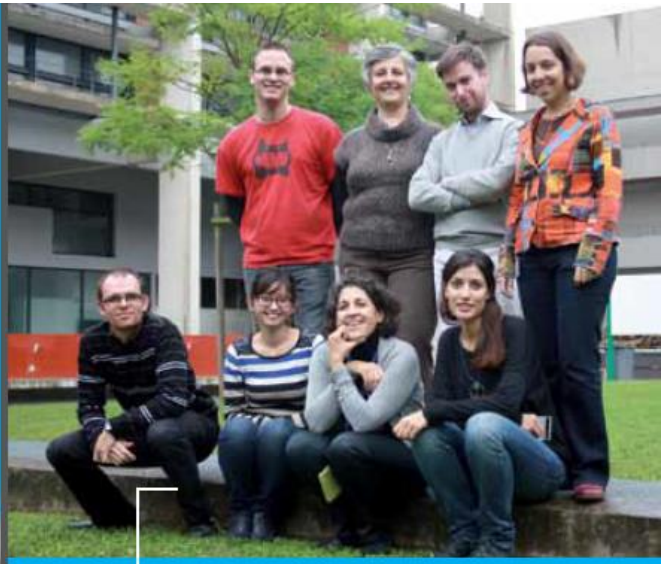
UNIVERSITÉ
de Cergy-Pontoise

PeptLab
UCP



PEPTLAB
Plateforme

Design,
Synthesis,
Purification and
Characterisation
of peptides and
proteins



Contacts

Scientific consultant
Prof. Anna-Maria Papini, PhD
Tél. : +33 6 65 65 16 68

Technical consultant
Olivier Monasson, PhD
Tél. : +33 1 34 25 70 68

peptlab@u-cergy.fr

Université de Cergy-Pontoise
Plateforme Peptlab@ucp
Site de Neuville
5 mail Gay-Lussac
Neuville-sur-Oise
95031 Cergy-Pontoise cedex
France

www.peptlab.eu